REMARKS/ARGUMENTS

Claims 1–25 are pending in the captioned application. Claims 1-10 and 19-24 have been withdrawn. Applicants have hereby cancelled claim 12 without prejudice. Claims 11, 13 -18 and 25 remain under examination in the instant action. Applicants have amended claims 13, 15-18 and 25 to bring them more fully in accordance with U.S. practice. Applicants have amended the specification and claim 13 in response to the Examiner's objections. Applicants have also amended claim 25, changing the single letter amino acid codes to three letter codes to conform to the sequence rules. Applicants have also amended claims 11 and 14 in response to the Examiner's rejections discussed below. Applicants respectfully submit the amendments are fairly based on the specification and respectfully request their entry.

The specification has been amended. The specification is objected to because "the sequence notification is written as "SEQ ID No. 4" instead of "SEQ ID NO: 4," and "the amino acids are reported using the one letter codes instead of the three letter codes." Applicants have corrected these errors. No new mater is added by these corrections.

Claims 12 and 13 are objected to because "the claims recite "SEQ ID No. 2" which is not the correct sequence notification." Applicants have amended claim 13 to

recite "SEQ ID NO: 2." Applicants have cancelled claim 12. Applicants respectfully assert the Examiner's objections should be withdrawn.

The Examiner has rejected claims 11-18 and 25 "under 35 U.S.C § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regards as the invention."

Specifically, the Examiner states, "Claims 11 and 14 and the dependent claims hereto are indefinite because the claim recites "and/or" and it is unclear if the slash mark represents "and", "or" or "and or"." In response, Applicants have amended claims 11 and 14 and deleted the slash marks.

In addition, the Examiner states, the claims "are indefinite because the residue positions are not tied to a reference sequence; therefore, it is unclear what is the protein that is only derived". The Examiner suggested that the limitations of claim 12 be incorporated into claim 11. In response, Applicants have amended claims 11 and 14, adding the sequence notification for the wild type GFP in each of the claims. Applicants also cancelled claim 12.

The Examiner continues, the "claims are also unclear as to: which one is the wild type and what is the difference in excitation and or emission spectrum." In response,

Applicants have amended claims 11 and 14 to clearly identify the wild type GFP sequence. In response, Applicants submit that the novel modified GFPs in the claimed invention exhibit clear differences in excitation and or emission spectrum as compared to the wild type GFP. These novel modified GFPs exhibit enhanced fluorescence relative to wtGFP when expressed in non-homologous cells at temperatures above 30°C. page 5, ll. 10 – 17. Detailed characterization of one of the modified GFPs in the current invention is presented in Example 4 of the application, and summarized in Table 2. This triple mutation F64L-S175G-E222G-GFP has an excitation peak (absorbance peak) of 481 nm, an emission peak of 506 nm. Wild type GFP, on the other hand, has a major excitation peak of 395-397 nm and a major emission peak of 504 nm, plus a minor excitation peak at 470-475 nm and a minor emission peak of 506 nm (Patterson et al. *Biophysical Journal*, 73, 2782-2790(1997). The triple mutations of the current application also show increased fluorescence intensity and delayed photobleaching, compared to wild type GFP (Fig. 5 and Fig. 6).

Thus, Applicants respectfully asserts that Examiner's rejection of claims 11-18 and 25 under 35 U.S.C § 112 cannot be sustained and should be withdrawn.

The Examiner has rejected claims 11-18 and 25 under 35 U.S.C. § 102(b) as being anticipated by Tsien et al. (WO96/23810, 8 August 1996). The Examiner states, "Tsien report a cDNA that encodes modified green fluorescent protein (GFP) with a point

mutation at position S65 ... to a Thr mutation ... having markedly different excitation and emission spectra from the corresponding products from wild type GFP." The Examiner continues, "Tsien disclose a DNA sequence that encodes the protein set forth in SEQ ID NO:2, 3 and 4 of the instant application and analogs thereof." The Examiner also asserts that Tsien teaches the expression of GFP in *E. coli*, fusion protein, expression vectors, host, as well as method of producing the protein. Applicants respectfully traverse this rejection for the reasons set forth herein.

Applicants submit that while Tsien et al. teach the sequence contained in SEQ ID NO: 2, it does not teach the sequence contained in SEQ ID NO: 3 or 4. The sequence disclosed in SEQ ID NO: 2 of the instant application is the prior art wild type GFP protein sequence (Figure 2), and does not anticipate the claimed inventions, nor does it anticipate SEQ ID NO: 3 or 4. Regarding claim 11 and 14, Applicants submit that the present composition as claimed requires amino acid substitution as three positions, namely position F64, S175 as well as one of S65 or E222. These triple mutations are not disclosed in Tsien et al., nor does Tsien et al. make any reference to a mutation at position 64, 175 or 222. Therefore, claim 11 or 14 is not anticipated by Tsien et al.. Thus, it is respectfully requested that the above rejection be withdrawn. Rejection of dependent claims 12-13, 15-18 and 25 should also be withdrawn, as these claims depend on Claim 11.

The Examiner has rejected claims 11-13 and 15-17 under 35 U.S.C. § 102(b) as being anticipated by Osumi et al. (U.S. Patent No. 6,194,548). The Examiner states, "Osumi et al. teach a DNA encoding GFP having mutations at positions S175, F64 and E222", "that exhibited different excitation spectrum". The Examiner continues, "Osumi et al. teach the sequences contained in SEQ ID NO: 2 and 3". The Examiner also states that Osumi et al. reports the use of an expression vector and expression in *E. coli*. Applicants respectfully traverse this rejection for the reasons set forth herein.

First, Applicants respectfully point out that the rejection under 35 U.S.C § 102(b) is improper. The Osumi et al. reference is a U.S. patent issued on February 27, 2001 (based on a patent application filed in July 1998), while the instant invention is filed September 28, 2001, and claims priority to a British application filed April 23, 2001.

Applicants aware that the Osumi et al. reference could be used as a prior art reference under 25 U.S.C § 102(a) or 102(e). Without admitting that Osumi et al. is prior art, Applicants submit that, even if it is applied under 35 U.S.C § 102(a) or 102(e), Osumi et al. does not anticipate the instant invention. Regarding claim 11, Applicants submit that the composition as claimed in the instant invention requires amino acid substitution as three positions, namely position F64, S175 as well as one of S65 or E222. These triple mutations are not disclosed in Osumi et al., nor does Osumi et al. make any reference to a mutation at position 222. Applicants also submit that while Osumi et al. teach the

sequence contained in SEQ ID NO: 2, it does not teach the sequence contained in SEQ ID NO: 3. The sequence disclosed in SEQ ID NO: 2 of the instant application is the prior art wild type GFP protein sequence (Figure 2), and does not anticipate the claimed inventions. Therefore, claim 11 is not anticipated by Osuni et al. It is respectfully requested that the above rejection be withdrawn. Rejection of dependent claims 12, 13 and 15-17 should also be withdrawn, as these claims depend on Claim 11.

The Examiner has rejected claims 11 and 15-18 under 35 U.S.C. § 102(e) as being anticipated by Bjorn et al. (WO 01/98338 19 June 2000). The Examiner states, "Bjorn et al. disclose DNA that encodes modified green fluorescent proteins (GFP) with a point mutation at position F64 and E222 having markedly different excitation and emission spectra from the corresponding products from wild type GFP". The Examiner also states that Bjorn et al. disclose COS7 as host cell, an expression vector, and a DNA construct comprising the vector. Applicants respectfully traverse this rejection for the reasons set forth herein.

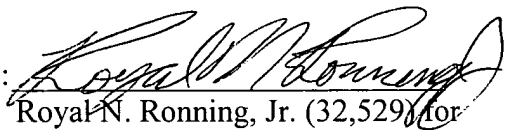
Regarding claim 11, Applicants submit that the present composition as claimed above, requires amino acid substitution at three positions, namely position F64, S175 as well as one of S65 or E222. These triple mutations are not disclosed in Bjorn et al., nor does Bjorn et al. make any reference to a mutation at position 175. In addition, the triple mutations of the instant application have far superior properties than the double mutations

of Bjorn et al. (Fig. 5, 6). Therefore, claim 11 is not anticipated by the Bjorn et al. reference. Thus, it is respectfully requested that the above rejection be withdrawn. Rejection of dependent claims 15-18 should also be withdrawn, as these claims depend on Claim 11.

Any questions with respect to the foregoing may be directed to Applicants' representative at the telephone number listed below.

Respectfully submitted,

AMERSHAM BIOSCIENCES CORP

By: 
Royal N. Ronning, Jr. (32,529) for
Yonggang Ji (53,073)
Agent for Applicants

Amersham Biosciences Corp
800 Centennial Avenue
P. O. Box 1327
Piscataway, New Jersey 08855-1327

Tel: (732) 980-2875
Fax: (732) 457-8463

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450, on January 13, 2004.

Signature: 

Name: Melissa Leck